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Research Article

IMMUNOMODULATORY ACTIVITY OF *CHATURTHAMALAKA RASAYANA*: AN EXPERIMENTAL EVALUATION

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ABSTRACT

Triphala, a simple combination of *Amalaki, Haritaki* and *Bibhitaki*, have proven antioxidant and immunomodulatory activities. But, work on *Chaturthamalaka Rasayana*, containing *Triphala*, under four alternate combinations, was not found reported yet. For this, a study was conducted to evaluate the effect of different four combinations under *Chaturthamalaka Rasayana* on leucocytes and immunoglobulin with special reference to immunomodulatory activity in albino wistar rat model. Four combinations of test drugs were prepared as per classical textual guidelines mentioned in Charaka Samhita. 36 wistar strain albino rats were used in the study. Immunosuppression done by Cyclophosphamide which induced neutropenia. Total leucocyte count (TLC) (cells/mm3), % Neutrophil count and Serum immunoglobulin level (in ZST units) were parameters used to evaluate. All the data were collected and analysed using paired't' test and one way ANOVA test, followed by Dunnett's multiple comparison 't' test. All the test formulations showed much better effect in comparison with the improvement noted in control group, as a cytoprotective agent. However, with respect to immunostimulation, the control group showed better effect in comparison to the test formulation. *Chaturthamalaka Rasayana* possesses significant cytoprotective activity and moderate immunostimulant activity. Among the four combinations, all test samples were found effective in immunosuppressive rats. Test sample IV containing *Amalaki, Haritaki* and *Bibhitaki* found more potent than or as similar as response in comparison of standard group (Levamisole). *Chaturthamalaka Rasayana* might be consider as a cost effective and adulteration free alternative formulation over used of costly *Chyavanaprasha Avaleha.*

KEYWORDS: Immunomodulation, *Rasayana, Chaturthamalaka, Ayurveda, Triphala.*

INTRODUCTION

From many decades, after successful laboratory research work, the whole world came to know about herbs to possess immunomodulatory properties. This action is done by stimulating both specific and nonspecific immunity. Many herbs used in Indian traditional system of medicine were reported to have immunomodulation activities. Some of these stimulate both humoral and cell mediated immunity while other activate only the cellular components of the immune system, i.e. phagocytic function without affecting the humoral or cell mediated immunity.

In *Ayurveda*, many traditional formulations are described as '*Rasayana*' have various pharmacological properties such as immuno-modulation, tonic, neurostimulant, antiaging, antibacterial, antiviral, anticancer, adoptogenic, antistress etc. An entire section of Materia Medica of Ayurveda is devoted to drugs entitled as '*Rasayana*' used for improvement of body resistance. *Rasayana Chikitsa* prevents the effects of early ageing develops intelligence and increase the body resistance against disease. In *Charaka Samhita*, there are many formulations under chapter *Rasayana Chikista Adhyaya*. Within this, after *Chyavanprasha Avaleha*,[1] *Chaturthamalaka Rasayana*[2] is described as a classical immunity booster formulation, beneficial to maintain immunity of an individuals. Now a days, *Chyavanprasha Avaleha* is the most popular *Ayurveda* formulation to the worldwide, which has too many numbers of contents, which are not easily available, may be in the form of adulterant due to *Churna*

(powder) form and also costly. As compared to that, *Chaturthamalaka Rasayana* has basic three main herbal ingredients in the form of fresh fruits of *Amalaki, Haritaki* and *Bibhitaki* with some another contents which are easily available. The *Phalashruti* (classical textual results) of *Chaturthamalaka Rasayana* explains the best *Rasayana Karma* which can be ultimately correlated with immunomodulation activity.[3]

On the base of the immunity status, recently, cancer has found as the leading cause of death in economically developed and the second leading cause of death in developed countries, among the non-communicable diseases.[4] For cancer, chemotherapy is the primary treatment available for disseminated malignant disease which acts by killing the cells that divides rapidly. This means chemotherapy also harms the cells that divide rapidly under normal circumstances in bone marrow.[5] That's why it ultimately results in to immunosuppression which is the major drawback of chemotherapy and also has the toxic side effects.[6] Cyclophosphamide is one of the most widely used broad spectrum antitumor agent, used in the treatment of cancer. Cyclophosphamide itself is a carcinogenic, lowers the body's ability to fight an infection causing Immunosuppression and also have side effects like bone marrow toxicity.[7] Hence in this study, Cyclophophamide is used as an immunosuppression agent in the animal model to validate the *Rasayana* effect. This is an attempt made to evaluate whether the four combinations of *Amalaki, Haritaki* and *Bibhitaki* under *Chaturthamalaka Rasayana* has the significant immunomodulatory action or not, also it was never been studied yet before. That's why, the present study designed to evaluate the effect of *Chaturthamalaka Rasayana* on leucocytes and immunoglobulin with special reference to immunomodulatory activity in albino wistar rat model.

MATERIALS AND METHODS

Test formulations

The formulations used for screening were test drug I (*Amalaki + Haritaki*), test drug II (*Amalaki + Bibhitaki*), test drug III (*Haritaki + Bibhitaki*) and test drug IV (*Amalaki + Haritaki + Bibhitaki*), prepared as per the classical textual method of *Charaka Samhita*. The whole work were conducted in two sections viz. toxicity evaluation and experimental study at Institute of biomedical and industrial research & Bilwal medchem research laboratory pvt ltd, Reengus, Jaipur, Rajasthan, India. All the trials were carried out after obtaining permission from Institutional animal ethics committee and Institutional ethics committee.

[A] Toxicity evaluation

Oral Acute Toxicity Study was done according to OECD guideline 423.

i) Animals and husbandry conditions

Healthy albino wistar rats (180-250 gm) of either sex were selected for the animal experiment and animals were maintained as per animal ethical committee regulations approved by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). They were acclimatized in the laboratory condition for two weeks prior to the experimentation. Animals had been group-caged on the bases of dose into group 1 to group 8 successively, each group have 3 rats.

ii) Preparation of animals

The animals were randomly selected, marked with Picric acid H (Mark on head), B (Mark on Back), T (Mark on Tail) for individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

iii) Number of animals and dose levels in toxicity study

Three animals were used for each group. The dose level to be used as the starting dose was selected from one of two fixed levels 2000 mg/kg body weight in four groups and 5000 mg/kg body weight in another four groups. Total eight groups were selected.

iv) Administration of doses

For toxicity studies, the test substance was administered in a single dose by gavage using an oral feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals weighed and the test substance administered. After the substance was administered, food was withheld for a further 3-4 hours in rats. All observations were systematically recorded.

[B] Experimental study

i) Animal grouping:

36 healthy adult albino wistar rats weighing between 180-250 gm were selected randomly and divided in 6 groups. Each group has 6 animals, treated for 63 days as shown in table 1.

Table 1: Grouping of animals for experimental study

Groups	Details	Day 1	Day 60	Day 63	Evaluation parameters
1.Normal Control	CMC (Vehicle) was given in this group	Blood sample collection from animals of all groups	Blood sample collection from animals of all groups + Cyclophosphamide (200 mg/kg, SC.) induced in animals of all groups	Blood sample collection from animals of all groups	➢ TLC number (cells/mm3) ➢ % Neutrophil number ➢ Serum immunoglobulin level (in ZST units)
2.Standard Control	Levamisole was given in this group				
3.Test drug I	Therapeutic dose with vehicle was given				
4.Test drug II	Therapeutic dose with vehicle was given				
5.Test drug III	Therapeutic dose with vehicle was given				
6.Test drug IV	Therapeutic dose with vehicle was given				

ii) Neutropenia causing (alkylating) agent

Cyclophosphamide injection (Brand name: Cycloxan 200mg), Batch No. KB7131001, MFG.-OCT.2016, EXP.-SEP.2020, Manufactured in India by Biochem pharmaceuticals Industry, Mumbai.

iii) Dose Fixation

The dose of the drug was calculated by extrapolating the therapeutic dose of human to rat dose on the basis of surface area ratio by referring to the table of *Paget and Barnes*.[8]

(a) *Chaturthamalaka Rasayana* as test drug

Suggested human dose for *Avaleha*[9] = 10gm BD

Rat dose = 10,000× 0.018×5 (Conversion factor).

= 900mg/kg BD

This dose was introduce in animals only after adding *Dadhi* in an equal quantity as same as, each of the other raw drugs taken in the formulation of *Chaturthamalaka Rasayana*, mentioned previously.

(b) Cyclophosphamide = Single dose of 200 mg//kg[10]

(c) Levamisole = 2.5mg/Kg [10]

Levamisole is an antiparasitic drug that also has been found immunomodulatory activity by enhancing T-cell function and cellular immunity (increase TLC number).

(**d) Normal Control** = 0.5% w/v CMC [Carboxymethylcellylose] solution, 1 ml/kg/day as a vehicle.

iv) Route of Administration

All the test drugs, vehicle and levamisole were administered orally with the help of oral feeding needle. Only Cyclophosphamide was given subcutaneously.

v) Experimental study

1) **Cyclophosphamide induced neutropenia**[10]

The Cyclophosphamide induced neutropenia model concentrates on the effect of drugs on the haemopoetic system. If the test drug causes decrease in the Cyclophosphamide induced neutropenia suggesting that it attenuates the effect of Cyclophosphamide on the haemopoetic system.

2) **Effect on Serum immunoglobulin level by using zinc sulphate turbidity (ZST) test**[10]

The estimation of serum immunoglobulin levels was used to evaluate the increase in serum immunoglobulin production after the administration of the drugs. Immunoglobulins are antibodies that react specifically with the antigen. The zinc sulphate turbidity test is used to gain a rough estimation of the amount of Immuno-globulins present in the serum. Zinc sulphate causes precipitation of the Immunoglobulins making the solution cloudy. A lack of cloudiness signifies lack of immunoglobulins. The turbidity is expressed as ZST units, which in turn indicate the amount of immunoglobulin present in the sample.

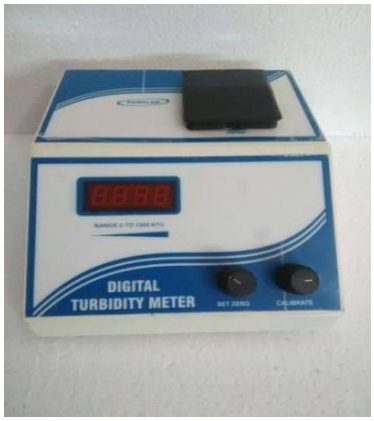
vi) Evaluation of blood On day 1st , 60th and day 63rd

a) Estimation of TLC (Total leucocyte count) number
b) Estimation of neutrophils
c) Estimation of immunoglobulin by zinc sulphate turbidity (ZST) test

vii) Study protocol (Fig 1)

First, the blood samples were collected on day 1, before administration of test drugs, from all the animals of all groups. Then the estimation of total leucocyte count (TLC) in cells/mm^3, neutrophil count and serum immunoglobulin level (in ZST units) in the blood was done. In the previously prepared test drug, the *Dadhi* was added in a quantity calculated according to dose calculations. Then all four test drugs along with vehicle control and standard control were administered for 60 days continuously. On 60th day, six hours after the last dose, again blood was collected for the estimation of the same previous blood parameters as same as done on day 1. On the same day (60th day), neutropenic dose of Cyclophosphamide was injected in animals of all groups that depletes expending lymphocyte population and this day was labelled as day Zero.[11] On day 3 (63rd day) after the injection of Cyclophosphamide, again blood was collected, for the estimation of the same previous blood parameters as same as done on day 1 and day 60. The serum was used for estimation of immunoglobulin levels using method devised by Mullen (1975).[12,13] Briefly, in this method, for each serum sample, a control tube containing 6 ml of distilled water and a test tube containing 6 ml of zinc sulphate solution was prepared. To each, 0.1 ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 hr at room temperature. The first tube served as blank and the second tube was taken as sample. The turbidity developed was measured using a Digital Nepheloturbidity Meter. The turbidity obtained (sample-blank) was compared with that obtained with standard barium sulphate (BaSO4) solution. The standard BaSO4 solution was prepared by adding 3 ml of barium chloride solution (1.15% w/v) to 97 ml of 0.2 N sulphuric acids. The turbidity obtained with this solution was expressed as 20 zinc sulphate turbidity (ZST) units. The TLC, neutrophil counts (%) and serum immunoglobulin level of the blood in treated groups were compared with the values of the control group.

Fig 1. Experimental work in animal laboratory

		
Rats Caging System	**Dose Administration**	**Blood Collection**
		
Equipment	**Oral Feeding Needle**	**ZST Test Apparatus**

viii) Statistical analysis

The experimental results of immunomodulatory effect of all four test drugs on TLC, neutrophils and serum Immunoglobulin level in wistar rats blood were expressed as mean ± S.E.M. Data were analysed by ordinary one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison 't' test using Graph pad Prism 7 software, with the level of significance set at $P < 0.05$ (at 95 % confidence interval).

OBSERVATIONS AND RESULTS

Toxicity study

Before starting actual experimental study, as per protocol, the toxicity study of all the four test samples was successfully done in animals. After administration of test drugs I, II, III, IV at dose 2000 mg/kg and 5000 mg/kg, it was found safe. No any behavioural and haematological changes found observed in 14 days.

Experimental Study

The results found in the animal study for four test drugs compared with control and standard drugs for 63 days were given below in table no 2 and 3, with graph no.1 for TLC number, in table no. 4 and 5, with graph no.2 for % neutrophil number and in table no.6 and 7, with graph no.3 for serum immunoglobulin level (in ZST units), separately for each parameter, where NS = Non significant, */**/*** = Significant and **** = Highly significant.

Table 2: Readings for TLC number (cells/mm3)

Days	Control	Standard	Test d. I	Test d. II	Test d. III	Test d. IV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
0 day	5474.67 ± 65.43	5457.50 ± 87.59	5429.67 ± 92.75	5568.33 ± 56.80	5311.17 ± 111.67	5400.17 ± 70.65
60th day	5423.00 ± 71.62	5555.33 ± 96.40	5471.83 ± 136.16	5560.67 ± 62.67	5672.00 ± 81.65	5758.50 ± 60.83
63rd day	2442.50 ± 67.12	3282.00 ± 76.71	3029.17 ± 42.08	2736.17 ± 49.58	2749.83 ± 52.73	3136.8 ± 35.08

Graph No.1: Readings for TLC number (cells/mm3)

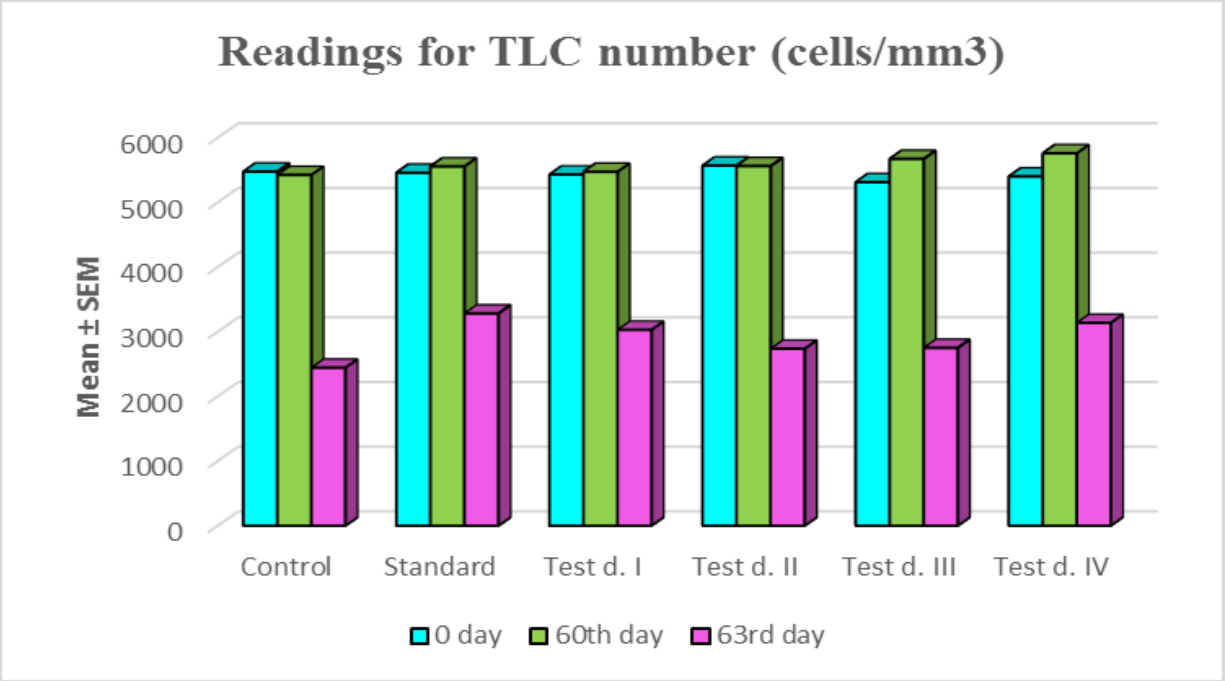


Table 3: Readings for TLC number (cells/mm3)

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
0 Day				
Control vs. Standard	17.17	-262 to 296.3	**NS**	**0.9997**
Control vs. Test I	45	-234.1 to 324.1	**NS**	**0.9929**
Control vs. Test II	-93.66	-372.8 to 185.5	**NS**	**0.8599**
Control vs. Test III	163.5	-115.6 to 442.6	**NS**	**0.4268**
Control vs. Test IV	74.5	-204.6 to 353.6	**NS**	**0.9384**
60th day				
Control vs. Standard	-132.3	-411.5 to 146.8	**NS**	**0.6247**
Control vs. Test I	-48.83	-328 to 230.3	**NS**	**0.9896**
Control vs. Test II	-137.7	-416.8 to 141.5	**NS**	**0.5896**
Control vs. Test III	-249	-528.1 to 30.15	**NS**	**0.0965**
Control vs. Test IV	-335.5	-614.6 to -56.35	*	**0.0123**
63rd Day				
Control vs. Standard	-839.5	-1119 to -560.4	****	**0.0001**
Control vs. Test I	-586.7	-865.8 to -307.5	****	**0.0001**
Control vs. Test II	-293.7	-572.8 to -14.52	*	**0.0356**
Control vs. Test III	-307.3	-586.5 to -28.18	*	**0.0255**
Control vs. Test IV	-694.3	-973.5 to -415.2	****	**0.0001**

Above table shows the data related to effect of all four test drugs along with standard and control drug on Total leucocyte count (TLC) in albino rats. After drug administration, on 60th day, it was found that only test drug IV increase the TLC level while others still remains same, with no better marginal differences. Administration of cyclophosphamide leads to the decrease in the count of TLC, especially neutrophils (neutropenia), confirms the reported myelosuppressive activity of the toxicant. After induction of neutropenia, on 63rd day, treatment with test drugs I and IV have highly significantly while test drug II and III have significantly maintain the TLC level when compared with standard drug.

Table 4: Readings for % Neutrophil number

Days	Control	Standard	Test d. I	Test d. II	Test d. III	Test d. IV
	Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
0 Day	14.34 ± 0.25	13.67 ± 0.22	14.03 ± 0.32	14.17 ± 0.26	14.04 ± 0.19	14.01 ± 0.24
60th day	14.42 ± 0.25	13.58 ± 0.28	15.75 ± 0.40	14.29 ± 0.29	14.29 ± 0.19	14.17 ± 0.20
63rd Day	6.43 ± 0.39	9.22 ± 0.30	8.00 ± 0.22	7.83 ± 0.19	7.91 ± 0.20	8.01 ± 0.44

Graph No.2: Readings for % Neutrophil number

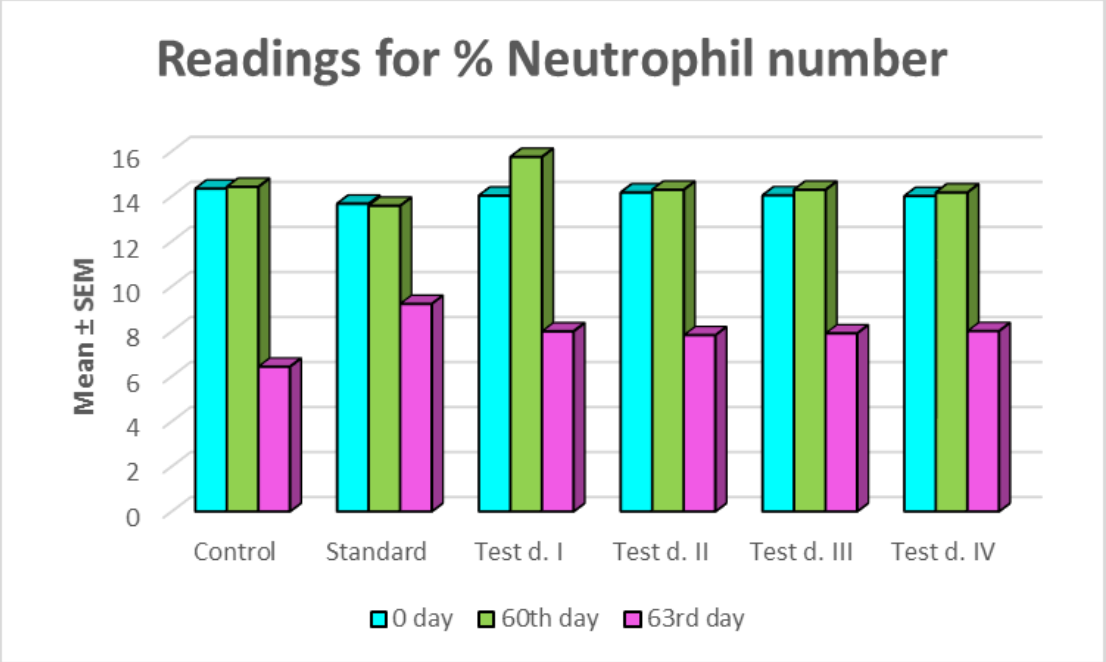


Table 5: Readings for % Neutrophil number

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
0 Day				
Control vs. Standard	0.67	-0.3376 to 1.678	**NS**	**0.3079**
Control vs. Test I	0.31	-0.6976 to 1.318	**NS**	**0.8959**
Control vs. Test II	0.17	-0.8376 to 1.178	**NS**	**0.9913**
Control vs. Test III	0.3	-0.7076 to 1.308	**NS**	**0.9075**
Control vs. Test IV	0.33	-0.6776 to 1.338	**NS**	**0.8708**
60th day				
Control vs. Standard	0.84	-0.1676 to 1.848	**NS**	**0.1340**
Control vs. Test I	-1.33	-2.338 to -0.3224	**	**0.0050**
Control vs. Test II	0.13	-0.8776 to 1.138	**NS**	**0.9971**
Control vs. Test III	0.13	-0.8776 to 1.138	**NS**	**0.9971**
Control vs. Test IV	0.25	-0.7576 to 1.258	**NS**	**0.9537**
63rd Day				
Control vs. Standard	-2.79	-3.798 to -1.782	****	**0.0001**
Control vs. Test I	-1.57	-2.578 to -0.5624	***	**0.0007**
Control vs. Test II	-1.4	-2.408 to -0.3924	**	**0.0028**
Control vs. Test III	-1.48	-2.488 to -0.4724	**	**0.0014**
Control vs. Test IV	-1.58	-2.588 to -0.5724	***	**0.0006**

Above table shows the data related to effect of all four test drugs along with standard and control drug on neutrophil count in albino rats. After drug administration, on 60th day, it was found that only test drug I increases the neutrophil count while others still remains same, with no better marginal differences. After induction of neutropenia, on 63rd day, treatment with all the test drugs have significantly maintain the level of neutrophil count when compared with standard drug.

Table 6: Reading for Serum immunoglobulin level (in ZST units)

Days	Control	Standard	Test I	Test II	Test III	Test IV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
0 Day	28.94 ± 0.50	28.84 ± 0.44	28.27 ± 0.55	28.60 ± 0.35	28.96 ± 0.43	28.87 ± 0.45
60th day	28.68 ± 0.46	28.47 ± 0.36	29.52 ± 0.45	28.91 ± 0.53	28.80 ± 0.42	28.57 ± 0.52
63rd Day	16.00 ± 0.39	23.29 ± 0.48	18.38 ± 0.78	17.86 ± 0.46	17.62 ± 0.55	19.71 ± 0.28

Graph No.3: Reading for Serum immunoglobulin level (in ZST units)

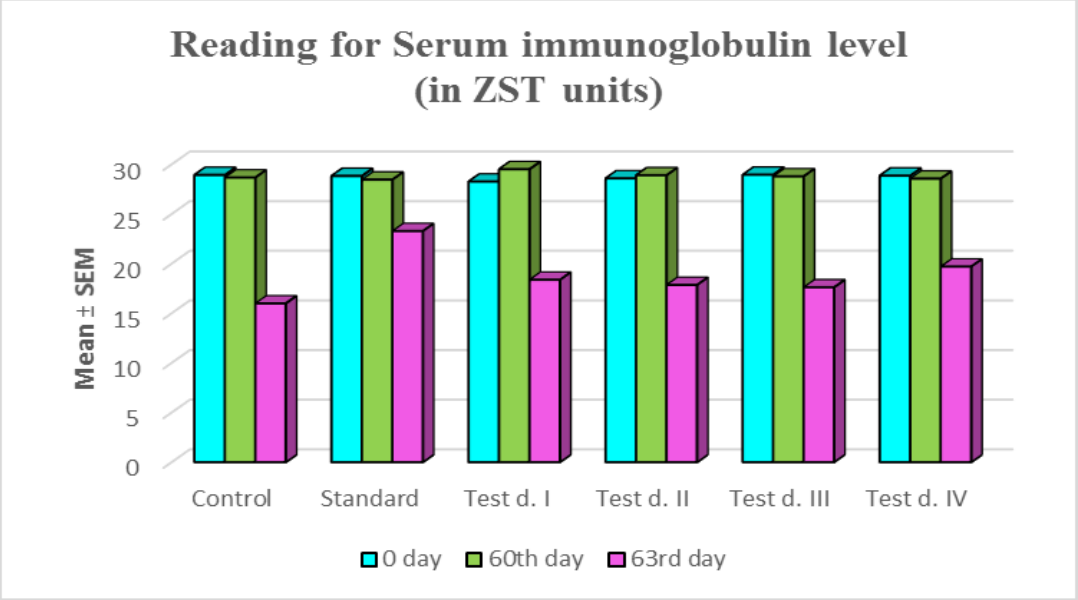


Table 7: Reading for Serum immunoglobulin level (in ZST units)

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
0 Day				
Control vs. Standard	0.1	-1.629 to 1.829	NS	0.9998
Control vs. Test I	0.67	-1.059 to 2.399	NS	0.7797
Control vs. Test II	0.34	-1.389 to 2.069	NS	0.9824
Control vs. Test III	-0.02	-1.749 to 1.709	NS	0.9999
Control vs. Test IV	0.07	-1.659 to 1.799	NS	0.9999
60th day				
Control vs. Standard	0.21	-1.519 to 1.939	NS	0.9980
Control vs. Test I	-0.84	-2.569 to 0.8892	NS	0.6033
Control vs. Test II	-0.23	-1.959 to 1.499	NS	0.9966
Control vs. Test III	-0.12	-1.849 to 1.609	NS	0.9997
Control vs. Test IV	0.11	-1.619 to 1.839	NS	0.9997
63rd Day				
Control vs. Standard	-7.29	-9.019 to -5.561	****	0.0001
Control vs. Test I	-2.38	-4.109 to -0.6508	**	0.0031
Control vs. Test II	-1.86	-3.589 to -0.1308	*	0.0303
Control vs. Test III	-1.62	-3.349 to 0.1092	NS	0.0741
Control vs. Test IV	-3.71	-5.439 to -1.981	****	0.0001

Above table shows the data related to effect of all four test drugs along with standard and control drug on serum immunoglobulin level in albino rats. After drug administration, on 60th day, it was found that none of the four test drug increases the serum immunoglobulin level, with better marginal differences as compared to standard drug. After induction of neutropenia, on 63rd day, treatment with test drug IV have highly significantly, test drugs I and II have significantly maintain the serum immunoglobulin level while test drug III have not maintain the level, with better marginal differences, as compared with standard drug.

DISCUSSION

[A] Toxicity Study

The alternate combinations of *Amalaki*, *Haritaki* and *Bibhitaki* in *Chaturthamalaka Rasayana* were found totally safe in experimental animals. None of the ingredients of the test drug has reported any toxicity. Further acute and chronic toxicity studies conducted on this basic drugs itself proves their safety profile as well.[14-19] Also all four test drugs were just a classical formulations made by logical thinking of great ancient experts. That's why prediction of not finding the chances of toxicity was absolutely proven true.

[B] Experimental Study

In animal experimental study, the results found were so encouraging to take the *Chaturthamalaka Rasayana* in a further clinical study. For more elaboration, the obtained pharmacological profile has been given in the form of final consolidated statement in table 8.

Table 8: Consolidated statement of effect of test formulations on parameters

Test Drug	TLC number (cells/mm³)			% Neutrophil number			Serum immunoglobulin (ZST units)		
	0 Day	60th Day	63rd Day	0 Day	60th Day	63rd Day	0 Day	60th Day	63rd Day
I	NS	NS	****	NS	**	***	NS	NS	**
II	NS	NS	*	NS	NS	**	NS	NS	*
III	NS	NS	*	NS	NS	**	NS	NS	NS
IV	NS	*	****	NS	NS	***	NS	NS	****

NS = Non Significant, */**/***= Significant, ****= Highly Significant

i) Effect on TLC number (cells/mm³)

On 60th day, only the test drug IV shows significant results and another sample had non-significant response. While after administration of immunosuppressant (Cyclophosphamide) and tests drugs for 3 days, it was found that all test drugs I, II, III, IV shows statistically significant changes in number of TLC. Test drug IV and after that test drug I had found similar response in comparison of standard group.

ii) Effect on % Neutrophil number

On 60th day, evaluation of immunomodulatory response on healthy wistar rats after oral administration of 900 mg/kg and observed neutrophils count was found that, test drug I had significant changes (p=0.0050). After administration of cyclophosphamide, on 63rd day, all the test drugs shows positive changes but found statistically less significant in comparison of standard.

iii) Effect on Serum immunoglobulin

On 60th day, evaluation of immunomodulatory response on healthy wistar rats after oral administration of 900 mg/kg and observed serum immunoglobulin level was found no any statically significant changes for all test drugs. After administration of immunosuppressant (Cyclophosphamide) and administration of tests samples for 3 days was found that, test drug IV had found similar response in comparison of standard group (p=0.0001).

[C] Probable mode of action of drug

A drug (*Dravya*) performs certain actions (*Karma*) in the body by virtue of its properties i.e. *Guna* (property), *Rasa* (taste), *Virya* (potency), *Vipaka* (metabolism) and *Prabhava* (exceptional activity) which exist in it in a state of co-inherence i.e. *Rasapanchaka* (table 9). Equality of proto-elements of the drug on one hand and the proto-elemental constituents of the body on the other hand forms on the basis of the *Samanya - Vishesha Siddhanta* (principles). These principles indicate that the leading proto-elements of the drug will increase similar proto-elements in the body and the dissimilar will decrease the proto-elements. The actions of

a drug are closely linked to its chemical structure in the form of a preponderance of one or two proto-elements presents to them.

Table 9: *Rasapanchaka* of contents of *Chaturthamalaka Rasayana*

S. No.	*Dravya*	*Rasa*	*Vipaka*	*Virya*	*Guna*	*Karma/Prabhava*
1.	*Amalaki*	*Pancharasa (Lavana Varjita)*	*Madhura*	*Shita*	*Guru, Ruksha*	*Rasayana* [20]
2.	*Haritaki*	*Pancharasa (Lavana Varjita)*	*Madhura*	*Ushna*	*Laghu, Ruksha*	*Rasayana* [21]
3.	*Bibhitaki*	*Kashaya*	*Madhura*	*Ushna*	*Laghu, Ruksha*	*Netrya, Keshya, Vaisvaryanashana* [22]
4.	*Dadhi*	*Amla, Kashaya*	*Amla*	*Ushna*	*Guru, Snigdha*	*Dipana, Hridya, Pushtikrita* [23]
5.	*Madhu*	*Madhura, Kashaya*	*Katu*	*Shita*	*Laghu, Ruksha*	*Yogavahi, Chedana, Lekhana* [24]
6.	*Ghrita*	*Madhura*	*Madhura*	*Shita*	*Guru, Snigdha*	*Rasayana, Dipana, Netrya* [25]
7.	*Tila*	*Katu, Tikta, Madhura, Kashaya*	*Katu*	*Ushna*	*Guru, Snigdha*	*Balya, Keshya, Agnivardhaka, Medhya* [26]
8.	*Tila Tailam*	*Madhura, Kashaya, Tikta*	*Madhura*	*Ushna*	*Guru, Snigdha*	*Bala Varnakaraka, Vrishya, Dipana* [27]
9.	*Sharkara*	*Madhura*	*Madhura*	*Shita*	*Laghu, Snigdha*	*Brihana, Vata-Rakta Pitta-Daha Shamana, Netrya* [28]

The *Rasayana Dravyas*, mostly those with *Madhura Vipaka* are advocated as 'adaptogen' in *Ayurveda*, primarily activate immune cells, leading to secretion of cytokines, which in turn act on multiple target organs. It has been found that the nervous, endocrine and immune systems are all interrelated. Immune products like various cytokines have been found to stimulate the hypothalamus-pituitary-adrenal axis and corticotrophin release factor (CRF), which ultimately enhances the production of adrenal corticotropic hormone (ACTH) resulting into increased secretion of glucocorticoids which have an overall suppressive effect on the immune system. Stress, anxiety and immunosuppression also act on the same axis and bring about changes in the immune status of the body. These *Rasayana Dravya* probably pretend to reduce stress levels of both body and mind. So these *Rasayana Dravya* act as a potent immunomodulator to keep health of a consumer.

In most human diseases, the oxidative stress is the secondary phenomena, for e.g., activated neutrophils produce O_2-, H_2O_2 and HOCl to kill phagogens. If a large number of phagocytes become activated in a localized area they can produce tissue damage. e.g., synovial fluid in sole and knee joints of rheumatoid arthritis contains large number of activated neutrophils. Some human diseases may be due to oxidative stress. e.g., excess radiation to biological system causes free radical damage to protein, DNA and lipids. Neurological disorders by dietary difference of tocopherol are mediative by oxidative stress. It also produces intracellular free Ca2+ damage to membrane ion transporters and other specific protein and peroxidation of lipids.

Hence, to protect from damage cells produce enzymes, or by intake of free radical scavenger substances to neutralizing or detoxify the free radical, known as antioxidant. Thus, the imbalance between free radical and anti-oxidant resulting diseases. In biological systems two types of antioxidants are proven useful against pathogenesis. Endogenous antioxidant and exogenous antioxidant. *Chaturthamalaka Rasayana* seems to be contained both types. The immune system has connections with a number of other organs and can directly or indirectly affect the actions of these organs. The function of the immune system is the protection of our bodies against foreign invaders. It plays a pivotal role in the pathogenesis of immune deficient diseases, autoimmunity and allergy. *Ayurveda* has great faith in pure treatment which cures the disease as well as provides physical, mental and social health too. *Rasayana* are health encouraging and rejuvenating agents which by their empirical effects produce resistance against disease both physically and mentally. Both lymphocytes and neutrophils were significantly increased by *Rasayana Chikitsa. Chaturthamalaka Rasayana* accelerated the recovery of the haemopoietic system by a rapid rise in total leukocytes. To induce neutropenia, we used

cyclophosphamide, a drug used in cancer treatment. It suggests that, the *Chaturthamalaka Rasayana* might be shows significant results in cancer patients.

CONCLUSION

The results of this experimental work are very encouraging & indicate that *Triphala* should be studied more extensively to confirm & reveal other potential therapeutic effects also. In toxicity study, all the test drugs I, II, III and IV under *Chaturthamalaka Rasayana* were discovered safe in acute dose upto 5000 mg/kg in albino rats. All the four test drugs were found effective in immunosuppressive rat model. Over all, *Chaturthamalaka Rasayana* is having significant cytoprotective activity and moderate immunostimulant activity. Among them, test drug IV containing *Triphala* found more potent, as similar as the standard group (Levamisole) having better activity profile in terms of both cytoprotective as well as immunostimulant activity. *Chaturthamalaka Rasayana* might be used as a cost effective and adulteration free *Rasayana* drug to overcome the use of *Chyavanprasha Avaleha*, which has issues of cost and adulteration. *Chaturthamalaka Rasayana* might be considered as an immunity booster drug in an adjuvant therapy. For this purpose, more clinical trials needed.

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